BIOSPECIMEN RESPOSITORY ACCESS & DATA SHARING
Division *of* Epidemiology, Statistics *and* Prevention Research

Datafile Documentation

NOTE: This is a preliminary and incomplete file. Additional biospecimens are still being analyzed and the results will be posted as soon as they are received from the investigators.

Material Source: _Collaborative Perinatal Project_____

Project: __Vitamin D: a link to racial disparities in adverse birth outcomes_____

Laboratory Assessment (s): 25-hydroxyvitamin D; high-sensitivity C-reactive protein; polymorphisms in vitamin D metabolizing genes

Component Description (Study, Grant, or Contract #)	Measurement of 25-hydroxyvitamin D [25(OH)D] and high-sensitivity C-reactive protein (CRP) from stored sera specimens. DNA will be extracted from stored sera and women and fetuses genotyped.
Eligible Sample	We selected our case and comparison groups among a sub-cohort of pregnancies that satisfied the following eligibility criteria: (1) singleton gestation, (2) no preexisting medical conditions (i.e., diabetes, hypertension, cardiovascular disease), (3) study entry at ≤26 weeks, and (4) maternal serum sample at ≤26 weeks that is available and can be retrieved. In this subcohort, all of these cases were selected. The group used as the comparison cohort was chosen by randomly selecting 11% of women the sub-cohort. Preeclampsia cases will be those with new-onset gestational hypertension and proteinuria, and return of abnormalities to normal in

	the postpartum period. Gestational hypertension was defined as two or more measurements of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg for the first time after 24 weeks. In the intrapartum period, the first five pressures obtained after hospital admission for delivery were averaged. Proteinuria was defined as two random urine dipsticks of 1+ protein, one dipstick of 2+ protein, or one intrapartum dipstick of 3+. Spontaneous preterm birth was defined as delivery of a liveborn infant at 26 to <35 completed weeks' gestation occurring after spontaneous preterm labor with intact membranes or spontaneous prelabor rupture of the fetal membranes, and infant birth weight <90th percentile based on a sonographically documented newborn size standard. 25(OH)D measured in maternal and cord serum; CRP measured in maternal serum.
Material (e.g., serum, urine)	Serum
Description of Laboratory Methods	25(OH)D: Serum 25(OH)D is the clinical indicator of vitamin D nutritional status [1,2]. Serum will be sent to Dr. Michael Holick at Boston University for analysis of total 25(OH)D ($25(OH)D_2$ + $25(OH)D_3$) using liquid-chromatography-tandem mass spectrometry [3]. Serum samples will be prepared and analyzed through a turbulent flow LC system followed by traditional laminar flow chromatography and are then be analyzed relative to the control solutions for detection and quantification of the 25(OH)D. The analysis will be performed using a TSQ Quantum Ultra triple mass-spectrometer (Thermo Finnigan Corp., San Jose, CA). The intra-assay coefficient of variation is 6.0%. High-sensitivity C-reactive protein. CRP is a reliable index of chronic systemic inflammation [4]. Serum high-sensitivity CRP will be measured in the laboratory of Dr. Simhan at the Magee-Womens Research Institute using ELISA kits. The detection limit of the CRP assay is 0.2 ng/ml, with intra- and inter-assay variabilities of 4% and 7%, respectively. Genotyping. We will study genes whose protein products are directly involved in the metabolism of vitamin D including: 25-hydroxylase (*CYP27A1*), 1a-hydroxylase (*CYP27B1*), vitamin D binding protein (*GC*), 24-hydroxylase (*CYP24A1*), vitamin D receptor (*VDR*), and retinoic acid receptor alpha (*RARA*). All methods will be carried out in the molecular epidemiology laboratory of Joseph Zmuda at the University of Pittsburgh Graduate School of Public Health. We will isolate high molecular weight genomic DNA from cells concentrated from serum aliquots using the QiaAMP DNA Kit (QIAGEN Inc., Valencia, CA). We will "bulk up" the DNA using the multiple

	displacement amplification method of whole genome amplification [5] with the REPLI-g kit (Qiagen) [6]. Amplified DNA will be quantified by the PicoGreen method. We will use the Sequenom MassARRAY iPLEX Gold platform as the primary method for genotyping of VDR SNPs. We have extensive experience using this platform and have found excellent call rates and reproducibility of genotype calls. The iPLEX Gold assay combines the benefits of robust single-base primer extension biochemistry with the sensitivity and accuracy of MALDI-TOF mass spectrometry detection. The iPLEX® Gold assay is based on multiplex PCR followed by a single base primer extension reaction. After PCR, remaining nucleotides are deactivated by SAP treatment. A single base primer extension step is performed, and the primer extension products are analyzed using MALDI-TOF mass spectrometry and cluster analysis and genotype calling are completed using Typer Software.
Laboratory Quality Control (QC) and Monitoring	See description above.
Data Processing and Editing	Samples were measured only once for each analyte (no replicates). Data were received after all the laboratory testing was complete. Samples where the quantity was not sufficient (QNS) for analysis were set to missing. The data were not otherwise edited. Data Access: All data are publicly available.
Dataset Format and Variables **Codebook Attached** **___ Yes** **_x__ No**	Excel file: CPP Identification number Date of blood draw CRP (μg/ml) Maternal 25(OH)D3 (nmol/l) Maternal 25(OH)D2 (nmol/l) Maternal total 25(OH)D (nmol/l) Cord 25(OH)D3 (nmol/l) Cord 25(OH)D2 (nmol/l) Cord total 25(OH)D (nmol/l)

Analytic Notes	
References	1. Haddad JG, Stamp TC. Circulating 25-hydroxyvitamin D in man. Am J Med 1974; 57(1): 57-62. 2. Hollis BW. Assessment of vitamin D nutritional and hormonal status: what to measure and how to do it. Calcif Tissue Int 1996; 58(1): 4-5. 3. Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, Petruschke RA, Chen E, de Papp AE. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005; 90(6): 3215-24. 4. Kluft C, de Maat MP. Sensitive markers of inflammation make it possible to study the chronic process: the rise of interest in low levels of C-reactive protein. Vascul Pharmacol 2002; 39(3): 99-104. 5. Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, Sun Z, Zong Q, Du Y, Du J, Driscoll M, Song W, Kingsmore SF, Egholm M, Lasken RS. Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci U S A 2002; 99(8): 5261-6. 6. Hosono S, Faruqi AF, Dean FB, Du Y, Sun Z, Wu X, Du J, Kingsmore SF, Egholm M, Lasken RS. Unbiased whole-genome amplification directly from clinical samples. Genome Res 2003; 13(5): 954-64.